

University of Dundee

## Leveraging unique structural characteristics of WNK kinases to achieve therapeutic inhibition

Zhang, Jinwei; Deng, Xianming; Kahle, Kristopher T.

*Published in:*  
Science Signaling

*DOI:*  
[10.1126/scisignal.aaj2227](https://doi.org/10.1126/scisignal.aaj2227)

*Publication date:*  
2016

*Document Version*  
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

### *Citation for published version (APA):*

Zhang, J., Deng, X., & Kahle, K. T. (2016). Leveraging unique structural characteristics of WNK kinases to achieve therapeutic inhibition. *Science Signaling*, 9(450), 1-3. [pe3]. <https://doi.org/10.1126/scisignal.aaj2227>

### **General rights**

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## PHARMACOLOGY

# Leveraging unique structural characteristics of WNK kinases to achieve therapeutic inhibition

Jinwei Zhang,<sup>1</sup> Xianming Deng,<sup>2</sup> Kristopher T. Kahle<sup>3\*</sup>

The with-no-lysine (K) WNK kinases are master regulators of the Na<sup>+</sup>-(K<sup>+</sup>)-Cl<sup>-</sup> cotransporters, including the renal-specific NCC and NKCC2 cotransporters. The discovery of WNK463, an orally bioavailable pan-WNK kinase inhibitor that exploits unique structural properties of the WNK catalytic domain to achieve high affinity and kinase selectivity, illustrates a strategy of leveraging distinct kinase features to develop specific inhibitors and validates the genetic predictions of the in vivo pharmacology of WNK inhibition.

Protein kinases are pathogenically mutated in multiple human diseases, including cancer, neuropsychiatric disorders, inflammatory conditions, infectious diseases, and cardiovascular diseases (1). About one-third of all protein targets under investigation in the pharmaceutical industry are kinases; more than 30 kinase inhibitors are approved for clinical use; and dozens of other kinase inhibitors are actively under investigation in clinical trials (2). Despite this, the available armamentarium of clinically used kinase inhibitors covers less than 15% of the kinome, and these are used overwhelmingly for oncological conditions (2). Compared to cancer in which lack of treatment is often lethal, the bar is set much higher for kinase drug discovery in chronic conditions, because exquisite target selectivity and a minimum of side effects are required to compete with existing therapies (1).

Kinases of the WNK (with-no-lysine) family are different from other kinases, because of the unusual placement of the catalytic lysine residue in WNK isoforms (Lys<sup>233</sup> of WNK1) compared to the active site lysine in all other protein kinases (3). This peculiarity could theoretically be exploited to create WNK-specific ATP-competitive kinase inhibitors. Indeed, Yamada *et al.* exploited these unique structural features to conduct a high-throughput screen for inhibitors of WNK1 catalytic activity (4). They discovered the first orally bioavailable pan-WNK kinase inhibitor, WNK463, which exhibits both low nanomolar affinity and high kinase selectivity. By solving the x-ray crystal structure of WNK463 with the kinase-dead mutant WNK1 S382A at 1.65 Å resolution, Yamada *et al.* showed that WNK463 contacts the hinge region of the ATP binding site by

burrowing through a narrow tunnel to the back pocket of WNK1, which occurs because of the nonstandard placement of the catalytic Lys<sup>233</sup> in the glycine-rich loop (Fig. 1A).

Genetic inhibition of WNK kinases promotes blood pressure reduction by stimulating both diuresis and vasodilation; therefore, these kinases have garnered much attention as potential targets for the development of anti-hypertensive agents. Mutations in WNK1 (encoded by *PRKWINK1*) and WNK4 (encoded by *PRKWINK4*) cause an autosomal dominant form of hypertension that is also associated with hyperkalemia termed Gordon's syndrome or pseudohypoaldosteronism type II (PHAII; OMIM #614496) (5). The WNK kinases regulate blood pressure and electrolyte homeostasis by phosphorylating and activating two related Ste20-type kinases termed STE20/SPS1-related proline/alanine-rich kinase (SPAK) and oxidative stress-responsive kinase 1 (OSR1), collectively referred to as SPAK/OSR1. Activated SPAK/OSR1 phosphorylate and stimulate the activities of two related cation-Cl<sup>-</sup> cotransporters in the kidney in the aldosterone-sensitive part of the nephron, the Na<sup>+</sup>-Cl<sup>-</sup> cotransporter NCC and the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter NKCC2 (Fig. 1B). WNKs also regulate NKCC1 and KCC2, cation-Cl<sup>-</sup> cotransporters that are critical for establishing Cl<sup>-</sup> homeostasis in the nervous system and are implicated in multiple diseases characterized by neuronal excitability due to GABA disinhibition (6). These actions of the WNKs make them attractive candidates for the development of inhibitors to treat these diseases as well.

Yamada *et al.* tested WNK463 in a rat hypertension model (4). In spontaneously hypertensive rats, orally administered WNK463 significant-

ly decreased blood pressure, increased urine output, and reduced the phosphorylation of SPAK and OSR1. This proof-of-biology study is important, because it establishes the importance of the WNK kinase catalytic domain in blood pressure and electrolyte homeostasis and confirms predictions made by human and rodent genetics about the in vivo pharmacology of WNK kinase inhibition (7). Moreover, WNK463 will be an important research reagent that will help illuminate CCC regulation and the role of

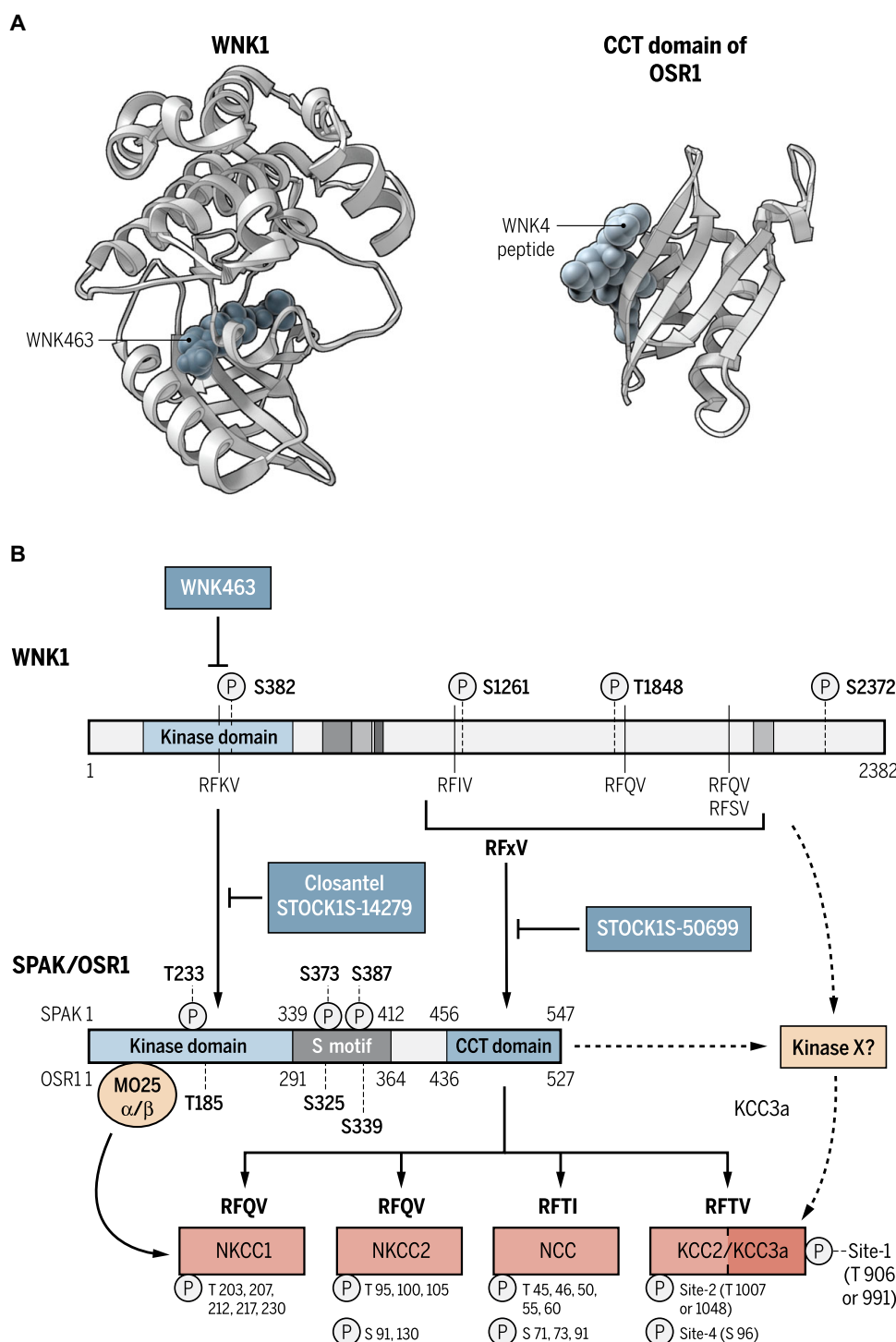
The development of WNK463 as a potential therapeutic was discontinued due to other unspecified effects beyond those reported in the cardiovascular and renal systems when administered to the rats at higher concentrations (4). This is perhaps not surprising, because the WNK kinases (including WNK2 and WNK3, kinases that are not mutated in PHAII) are present throughout the body, although some isoforms exhibit restricted distribution. For example, the HSN2 isoform of WNK1 is almost exclusively detected in the nervous system, including the spinal cord dorsal horn, and inactivating HSN2 mutations cause hereditary sensory and autonomic neuropathy type IIA (HSANII; OMIM #201300) (8). Pan-WNK inhibition might, therefore, affect physiological process beyond those of blood pressure and electrolyte homeostasis.

With regard to alternative means to inhibit the WNK pathway, where to go from here? The WNK substrates SPAK and OSR1 play a critical role in controlling blood pressure, and SPAK-deficient mice have markedly reduced blood pressure yet are otherwise healthy (9). Genome-wide association studies of essential hypertension show a strong association with common variants of SPAK. The strategy of targeting the ATP binding site of SPAK/OSR1 raises concern regarding the ability to develop sufficiently selective inhibitors that do not suppress other kinases. The development of Closantel and STOCK1S-14279, ATP-insensitive inhibitors,

2016 © The Authors,  
some rights reserved;  
exclusive licensee  
American Association  
for the Advancement  
of Science.

<sup>1</sup>MRC Protein Phosphorylation and Ubiquitylation Unit, College of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland. <sup>2</sup>State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, Xiamen, Fujian 361102, China. <sup>3</sup>Department of Cellular and Molecular Physiology and Centers for Mendelian Genomics, Yale School of Medicine, New Haven, CT 06510, USA.

\*Corresponding author. Email: kristopher.kahle@yale.edu



**Fig. 1. Domains and sites important for regulation of and signaling through the WNK-SPAK/OSR1 pathway.** (A) Left: The structure of WNK463 bound to the kinase-dead mutant WNK1 S382A (PDB ID: 5DRB). Right: The structure of the OSR1 CCT domain bound to the RfxV motif from WNK4 (PDB ID: 2V3S). (B) Proteins with slashes indicate that multiple isoforms have the same properties. For SPK/OSR1, the residue numbering above the protein represents SPK, and the residue numbering below represents OSR1. Kinase X refers to a yet unidentified kinase that is regulated by WNKs and mediates the direct phosphorylation and inhibition of Site 1 on the KCCs. STOCK1S-50699 is a small-molecule inhibitor that blocks the interaction between SPK/OSR1 and WNK by binding to the CCT domain (13); Closantel and STOCK1S-14279 are SPK (ATP-insensitive) inhibitors that bind constitutively active or WNK-sensitive (T233E)SPK (10); and WNK463 inhibits WNK1 catalytic activity (4).

has introduced the possibility of developing inhibitors of the WNK pathway by binding to constitutively active or WNK-activated (T233E)SPK (Fig. 1B) (10).

Another approach is to target protein-protein interaction sites. SPK has a specific docking CCT domain that mediates the interaction with RfxV motifs present in WNKs, NCC, and NKCC2 (Fig. 1B) (11). A high-resolution three-dimensional structure of this domain complexed to the RfxV motif has been solved (Fig. 1A) (12). Screens have identified inhibitors that disrupt the CCT-RfxV interaction (13), and chemical modulation of these first-generation inhibitors might yield compounds that could be used in vivo. Because the CCT domain is unique to SPK and OSR1, targeting this interaction site would not be expected to inhibit other kinases, which could provide sufficient specificity and safety for use in the treatment of a chronic condition, such as hypertension. Other strategies that antagonize tissue-specific WNK isoforms, for example, by targeting the unique HSN2 isoform to treat neuropathic pain (14), could minimize unwanted side effects in other organ systems.

Major challenges remain for the development of therapeutically effective kinase inhibitors in non-oncological diseases, including the identification and validation of driver kinases in these conditions and the discovery of drugs with adequate selectivity and safety (2). Targeting kinases that, when mutated, disrupt human physiology (even in rare inherited forms of disease) is a good strategy for the development of personalized treatments (15). With eight members of the WNK kinase signaling pathway, including their upstream regulators (the E3 ubiquitin ligase complex CUL3-KLHL3) and downstream targets (SPK and OSR1 and cation-Cl<sup>-</sup> cotransporters), being mutated in Mendelian forms of renal and central nervous system pathology (7), continued efforts at drug discovery targeting this pathway are most certainly warranted.

## REFERENCES

1. P. Cohen, D. R. Alessi, Kinase drug discovery—What's next in the field? *ACS Chem. Biol.* **8**, 96–104 (2013).
2. D. Fabbro, 25 years of small molecular weight kinase inhibitors: Potentials and limitations. *Mol. Pharmacol.* **87**, 766–775 (2015).
3. X. Min, B.-H. Lee, M. H. Cobb, E. J. Goldsmith, Crystal structure of the kinase domain of WNK1, a kinase that causes a hereditary form of hypertension. *Structure* **12**, 1303–1311 (2004).

4. K. Yamada, H.-M. Park, D. F. Rigel, K. DiPetrillo, E. J. Whalen, A. Anisowicz, M. Beil, J. Berstler, C. E. Brocklehurst, D. A. Burdick, S. L. Caplan, M. P. Capparelli, G. Chen, W. Chen, B. Dale, L. Deng, F. Fu, N. Hamamatsu, K. Harasaki, T. Herr, P. Hoffmann, Q.-Y. Hu, W.-J. Huang, N. Idamakanti, H. Imase, Y. Iwaki, M. Jain, J. Jeyaseelan, M. Kato, V. K. Kaushik, D. Kohls, V. Kunjathoor, D. LaSala, J. Lee, J. Liu, Y. Luo, F. Ma, R. Mo, S. Mowbray, M. Mogi, F. Ossola, P. Pandey, S. J. Patel, S. Raghavan, B. Salem, Y. H. Shanado, G. M. Trakshel, G. Turner, H. Wakai, C. Wang, S. Weldon, J. B. Wielicki, X. Xie, L. Xu, Y. I. Yagi, K. Yasoshima, J. Yin, D. Yowe, J.-H. Zhang, G. Zheng, L. Monovich, Small-molecule WNK inhibition regulates cardiovascular and renal function. *Nat. Chem. Biol.* 10.1038/nchembio.2168 (2016).
5. F. H. Wilson, S. Disse-Nicodème, K. A. Choate, K. Ishikawa, C. Nelson-Williams, I. Desitter, M. Gunel, D. V. Milford, G. W. Lipkin, J.-M. Achard, M. P. Feely, B. Dussol, Y. Berland, R. J. Unwin, H. Mayan, D. B. Simon, Z. Farfel, X. Jeunemaitre, R. P. Lifton, Human hypertension caused by mutations in WNK kinases. *Science* **293**, 1107–1112 (2001).
6. K. T. Kahle, E. Delpire, Kinase-KCC2 coupling: Cl<sup>−</sup> rheostasis, disease susceptibility, therapeutic target. *J. Neurophysiol.* **115**, 8–18 (2016).
7. D. R. Alessi, J. Zhang, A. Khanna, T. Hochdörfer, Y. Shang, K. T. Kahle, The WNK-SPAK/OSR1 pathway: Master regulator of cation-chloride cotransporters. *Sci. Signal.* **7**, re3 (2014).
8. M. Shekarabi, N. Girard, J.-B. Rivière, P. Dion, M. Houle, A. Toulouse, R. G. Lafrenière, F. Vercauteren, P. Hince, J. Laganier, D. Rochefort, L. Faivre, M. Samuels, G. A. Rouleau, Mutations in the nervous system-specific *HSN2* exon of *WNK1* cause hereditary sensory neuropathy type II. *J. Clin. Invest.* **118**, 2496–2505 (2008).
9. B. P. Zambrowicz, A. Abuin, R. Ramirez-Solis, L. J. Richter, J. Piggott, H. BeltrandelRio, E. C. Buxton, J. Edwards, R. A. Finch, C. J. Friddle, A. Gupta, G. Hansen, Y. Hu, W. Huang, C. Jaing, B. W. Key Jr., P. Kipp, B. Kohlhauff, Z.-Q. Ma, D. Markesich, R. Payne, D. G. Potter, N. Qian, J. Shaw, J. Schrick, Z.-Z. Shi, M. J. Sparks, I. Van Sligtenhorst, P. Vogel, W. Walke, N. Xu, Q. Zhu, C. Person, A. T. Sands, Wnk1 kinase deficiency lowers blood pressure in mice: A gene-trap screen to identify potential targets for therapeutic intervention. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14109–14114 (2003).
10. E. Kikuchi, T. Mori, M. Zeniya, K. Isobe, M. Ishigami-Yuasa, S. Fujii, H. Kagechika, T. Ishihara, T. Mizushima, S. Sasaki, E. Sohara, T. Rai, S. Uchida, Discovery of novel SPAK inhibitors that block WNK kinase signaling to cation chloride transporters. *J. Am. Soc. Nephrol.* **26**, 1525–1536 (2015).
11. J. Zhang, K. Siew, T. Macartney, K. M. O'Shaughnessy, D. R. Alessi, Critical role of the SPAK protein kinase CCT domain in controlling blood pressure. *Hum. Mol. Genet.* **24**, 4545–4558 (2015).
12. F. Villa, J. Goebel, F. H. Rafiqi, M. Deak, J. Thastrup, D. R. Alessi, D. M. F. van Aalten, Structural insights into the recognition of substrates and activators by the OSR1 kinase. *EMBO Rep.* **8**, 839–845 (2007).
13. T. Mori, E. Kikuchi, Y. Watanabe, S. Fujii, M. Ishigami-Yuasa, H. Kagechika, E. Sohara, T. Rai, S. Sasaki, S. Uchida, Chemical library screening for WNK signalling inhibitors using fluorescence correlation spectroscopy. *Biochem. J.* **455**, 339–345 (2013).
14. K. T. Kahle, J.-F. Schmouth, V. Lavastre, A. Latremoliere, J. Zhang, N. Andrews, T. Omura, J. Laganier, D. Rochefort, P. Hince, G. Castonguay, R. Gaudet, J. C. S. Mapplebeck, S. G. Sotocinal, J. Duan, C. Ward, A. R. Khanna, J. S. Mogil, P. A. Dion, C. J. Woolf, P. Inquimbert, G. A. Rouleau, Inhibition of the kinase WNK1/HSN2 ameliorates neuropathic pain by restoring GABA inhibition. *Sci. Signal.* **9**, ra32 (2016).
15. R. P. Lifton, A. G. Gharavi, D. S. Geller, Molecular mechanisms of human hypertension. *Cell* **104**, 545–556 (2001).



10.1126/scisignal.aaj2227

**Citation:** J. Zhang, X. Deng, K. T. Kahle, Leveraging unique structural characteristics of WNK kinases to achieve therapeutic inhibition. *Sci. Signal.* **9**, pe3 (2016).

## Abstract

**One-sentence summary:** Exploiting unique characteristics of the WNK-SPAK pathway may yield useful antihypertensive medications.